

Effects of High-sugar and High-fat Diet on Fat Deposition and Blood Vessel Wall on Sprague Dawley Rats Liver

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Abstract

People nowadays tend to consume more fast food and sweetened beverages. These foods usually contain high amount sugar and fat that have effects on the body including liver. This study was conducted to explore the effects of extensive intake of sugar and fat on blood glucose and cholesterol level as well as changes in liver. Research was conducted with experimental method using 20 Sprague Dawley rats which were divided into 4 groups; 2 controls and 2 treatments. Rats were given 5 ml sugar or lard alternatively every 2 consecutive days for 1-month and 2-month respectively. Data was retrieved include blood glucose and cholesterol level, fatty liver percentage and blood vessel thickening after intervention through HE staining. The results showed that both 1-month and 2-month intervention group has significant increase in blood glucose and cholesterol level. However, the enhancement of fatty liver percentage and number of thickened blood vessels ($p < 0.05$) were only found significant ($p < 0.05$) in 1-month intervention group. We concluded that high intake of sugar and fat within 1-month intervention have significant effects on the rat body including liver. Nevertheless, it was not found significant in 2-months intervention. Further studies are still needed to analyze this incongruent result.

Keywords: high-sugar diet, high-fat diet, fatty liver, atherosclerosis

Efek Konsumsi Gula dan Lemak yang Berlebihan terhadap Deposisi Lemak dan Pembuluh Darah pada Hati Tikus Sprague Dawley

Abstrak

Kebanyakan orang pada saat ini cenderung mengonsumsi lebih banyak makanan siap saji dan minuman dengan pemanis rasa yang mengandung banyak gula dan lemak. Kondisi tersebut dapat menyebabkan berbagai kelainan dalam tubuh terutama di hati. Penelitian ini dilakukan untuk mengkaji efek konsumsi gula dan lemak yang berlebihan terhadap kadar glukosa dan kolesterol darah serta perubahan pada hati. Penelitian ini merupakan penelitian eksperimental dengan menggunakan 20 tikus jenis Sprague Dawley dibagi menjadi 4 grup; 2 kontrol dan 2 perlakuan. Subjek dicekok dengan 5 ml gula atau lemak secara bergantian setiap 2 hari selama 1 dan 2 bulan. Data yang dianalisis yaitu kadar glukosa dan kolesterol darah, persentase lemak hati dan penebalan dinding pembuluh darah. Hasil penelitian menunjukkan bahwa peningkatan yang signifikan ($p < 0.05$) pada kadar glukosa dan kolesterol darah ditemukan pada kedua kelompok perlakuan baik selama 1 bulan maupun 2 bulan. Namun, peningkatan persentase lemak hati dan penebalan dinding pembuluh darah ditemukan meningkat bermakna hanya pada kelompok perlakuan 1 bulan. Kesimpulan penelitian ini yaitu peningkatan intake gula dan lemak selama 1 bulan dapat menyebabkan gangguan pada tubuh tikus termasuk hati. Namun gangguan tersebut tidak ditemukan bermakna pada kelompok perlakuan 2 bulan. Penelitian lebih lanjut diperlukan untuk menganalisis perbedaan hasil tersebut.

Kata kunci: konsumsi gula tinggi, konsumsi lemak tinggi, perlemakan hati, atherosklerosis

Introduction

Food is primary needs of human that brings pleasure to them. While there are tons of foods with varying taste, people tend to choose delicious foods which is usually less healthy. As a matter of fact, over amount of food consumption can lead to certain health problems such as obesity and diabetes. Research in Indonesia shows an increasing number of oil and fat containing food consumption as well as sugar containing food consumption from time to time. A rise of 0.5% in sugar consumption has been reported within 9 years, from 4.7% in 1993 to 5.2% in 2002.¹ Oil and fat consumption increased significantly from 7.6% from all meal in 1993 to 10.2% in 2002.² In human body, high amount of sugar consumption can cause hyperglycemia while high amount of oil and fat consumption can cause hyperlipidemia.

It was known that excess intake of both lipid and sugar is known to cause not only

cardiovascular diseases, but also could lead to serious liver diseases as well as other metabolic syndromes.^{3,4} Nevertheless, liver problems induced by over consumptions have not attracted adequate attention in the society as the extent of damage of the liver has not been fully understood. Therefore, this research will be conducted to find out the effect of excess intake of lipid and sugar to the liver.

As there is an increase in the prevalence of fatty liver disease and arteriosclerosis worldwide, extensive assessment to all the risk factors is important to prevent further increase of the prevalence rate. In this research, we will find the association between high-sugar and high-lipid diet in causing thickening of vessels walls and fatty liver.

White sugar usually consists of mainly sucrose, a disaccharide that will eventually breakdown into glucose and fructose.^{5,6} Lard is a term used to describe fat obtained from pigs by melting and straining it from the cell wall tissues encasing it (Table 1).^{7,8}

Table 1. Comparative Properties of Common Cooking Fats (per 100g)⁷

Common cooking fats	Total fat	Saturated fat	Monounsaturated fat	Polyunsaturated fat	Protein
Vegetable shortening (hydrogenated)	71 g	23 g	8 g	37 g	0 g
Sunflower oil	100 g	10 g	84 g	4 g	0 g
Soybean oil	100 g	16 g	23 g	58 g	0 g
Olive oil	100 g	14 g	73 g	11 g	0 g
Lard	100 g	39 g	45 g	11 g	0 g
Butter	81 g	51 g	21 g	3 g	1 g

Hepatic steatosis, also known as fatty liver, is a condition commonly affecting the liver after intake of alcohol.^{9,10} Non-alcoholic fatty liver disease, a condition resembling alcohol-induced liver disease without heavy drinkers as a predisposing factor, affects both men and women equally. A strong relationship has been established among this disease and obesity, dyslipidemia, hyperinsulinemia, insulin resistance, as well as overt type 2 diabetes.¹¹

Arteriosclerosis is defined as thickening and elasticity loss of arterial walls. It can be classified into several types including atherosclerosis and arteriolosclerosis.¹² Atherosclerosis has a characteristic of intimal lesions known as atheromas or fibrofatty plaques protruding into and obstructing vascular lumens resulting in weaken underlying media.¹³

Hyperlipidemia mainly hypercholesterolemia is one of the major risk factor for atherosclerosis as increased levels of serum cholesterol alone are sufficient to trigger lesion development without other additional risk factors.¹² Increase dietary intake of cholesterol and saturated fats including egg yolk,

animals fats and butter will raise the plasma level of cholesterol and vice versa.^{10, 11}

Arteriolosclerosis is the term used when the thickening of vessel walls occur in small arteries and arterioles. There are 2 varieties of arteriosclerosis, hyaline and hyperplastic arteriosclerosis, in which both are associated with thickening of vessels wall concurrently with luminal narrowing resulting in downstream ischemic injury.¹⁰

Methods

The study design was experimental design and research was done in the Pathology Anatomy Laboratory of Faculty of Medicine, University of Indonesia between April and July 2009. Rats are grouped to 4 groups and each rat is given 5 ml of either liquefied sugar or dry-rendered lard once in two days for one or two months according to the group. Subjects used were Sprague Dawley male rats, age 3-4 months with weight 200-250 g.

According to the sample size calculation, the number of subjects needed was 24. Nevertheless,

during the experiment, only 20 rats were used as that was the number of rats available at that time.

1. Control group 1: given processed rat food every day for a month
2. Treatment group 1: given processed rat food every day with additional sugar solution or lard

every two days simultaneously for a month

3. Control group 2: given processed rat food every day for two months
4. Treatment group 2: given processed rat food every day with additional sugar solution or lard every two days simultaneously for two months

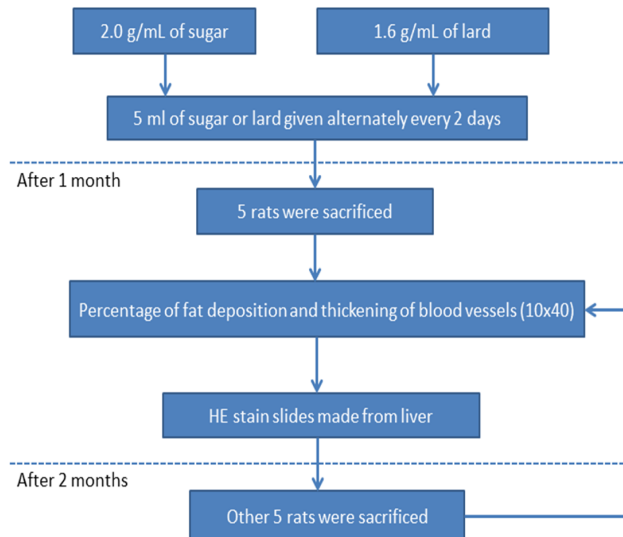


Figure 1. Flow-chart of the research procedure in this animal study

Sugar and lard were prepared accordingly with details described in Figure 1. Variables measured were blood sugar level and blood cholesterol level

as well as their correlation with the occurrence of blood vessel thickening and fatty liver. These were done by obtaining data of fatty liver percentage and the amount of thickened blood vessels walls.

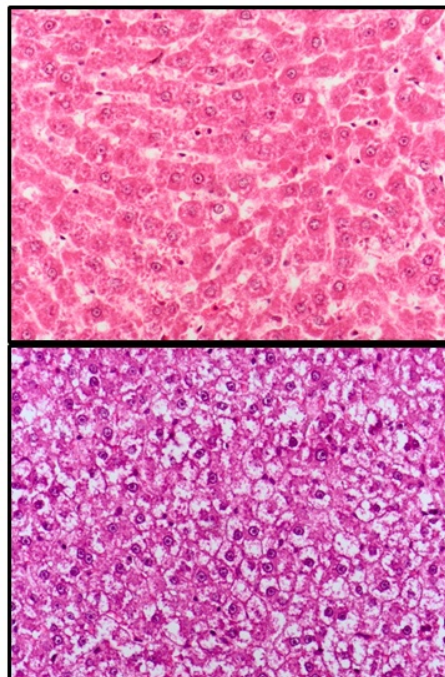


Figure 2. Liver cells in 10x40 magnifications in fatty liver percentage calculation; in control group (above); in treatment group (below)

To count the amount of fat deposition in liver tissue, 5 fields of 10x40 magnifications were taken in random. The percentage of fat deposition in liver tissue was calculated based on the pixels of the fat vacuoles in hepatocyte divided by the total pixels of a particular field. An example of the picture taken was shown in Figure 2.

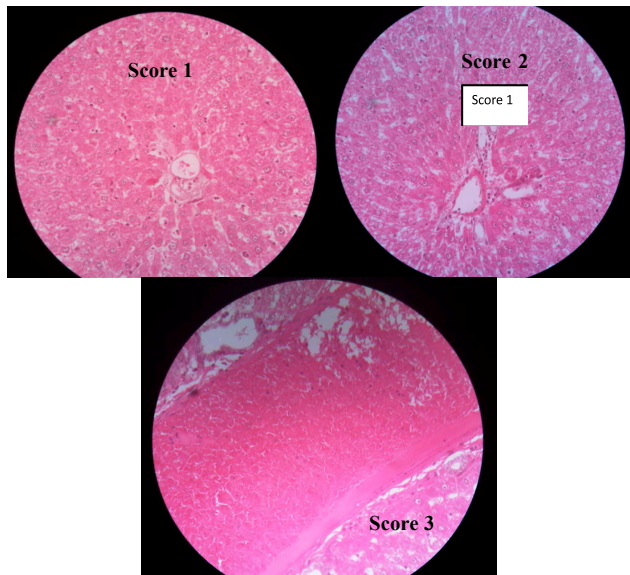


Figure 3. Different levels of blood vessels thickening

To count the thickening of vessels walls in liver tissue, 15 blood vessels were observed randomly, and the blood vessel walls were categorized according to the classification (Figure 3) as followed:

1. Score 1: if there is no thickening of blood vessels
2. Score 2 : if there is thickening of blood vessels
3. Score 3 : if there is thickening of blood vessels accompanied by aggregation formation

The score for each slide was noted based on the sum of the score for 15 blood vessels found in the slide.

In analyzing the data, SPSS software version 17.0 was used. First, normality test was performed to evaluate the distribution of the data. If the data is parametric and normally distributed then t-test and Pearson's correlation were used. If the data is categorical or metric that was not normally distributed then Wilcoxon Signed-Rank test, Mann-Whitney test and Spearman's correlation test were used.¹⁵

Results

Experiment results

Data obtained in this experiment include baseline and average data of blood glucose level, baseline and average data of blood cholesterol level, fatty liver percentage and thickening of blood vessels in all control and treatment groups.

Results on blood glucose level

In groups with a month intervention, the mean baseline data for blood glucose was slightly higher by 4.00 ± 3.67 mg/dL in control group compared to that of treatment group (97.80 ± 4.76 mg/dL vs. 93.80 ± 6.69 mg/dL). On the other hand, in the two months groups, the blood glucose data at baseline was marginally higher by 8.00 ± 6.11 mg/dL in treatment group (99.80 ± 6.57 mg/dL) than that of control group (91.80 ± 11.99 mg/dL). Based on the result of these blood analysis, the baseline data for glucose level in control groups compared to that of treatment groups in both a month and two months intervention were not significantly different (independent t-test; $p > 0.05$) (Table 2).

In both control groups, the blood glucose level decreased when compared from baseline data to average data, by 3.66 ± 3.86 mg/dL in control group 1 (97.80 ± 4.76 mg/dL to 94.14 ± 2.83 mg/dL) and by 9.62 ± 11.70 mg/dL in control group 2 (91.80 ± 11.99 mg/dL to 82.18 ± 1.48 mg/dL). However, these results were found to be not statistically significant (paired t-test; $p > 0.05$). In the treatment groups, on the other hand, the blood glucose level increased by 14.70 ± 7.03 mg/dL in 1-month intervention group (93.80 ± 6.69 mg/dL to 108.50 ± 1.31 mg/dL) and by 36.61 ± 22.65 mg/dL in 2-month intervention group (99.80 mg/dL to 136.41 ± 18.76 mg/dL). Both of these increase were statistically significant (paired t-test; $p < 0.05$) (Table. 2).

Following a-month intervention, there was a statistically significant difference between the treatment and control groups, where treatment group had higher outcome by 14.36 ± 1.40 mg/dL compared to that of control group (108.50 ± 1.31 mg/dL vs. 93.80 ± 6.69 mg/dL; independent t-test; $p < 0.05$). Similarly, in two-month intervention groups, the difference of blood glucose level after intervention between the treatment and control groups was also statistically significant. The treatment group had higher results by 54.23 ± 8.41 mg/dL compared to that of control group (136.41 ± 18.76 mg/dL vs. 99.80 ± 6.57 mg/dL; independent t-test; $p < 0.05$) (Table 2).

Table 2. Results of all variables at baseline and average (express in mean \pm SD)

Rat		Baseline		Average		p-value [#]
		Control	Treatment	Control	Treatment	
Blood glucose (mg/dL)	1	97.80 \pm 4.76	93.80 \pm 6.69	94.14 \pm 2.83	108.50 \pm 1.31	p<0.05
	2	91.80 \pm 11.99	99.80 \pm 6.57	82.18 \pm 1.48	136.41 \pm 18.76	p<0.05
Blood cholesterol (mg/dL)	1	165.20 \pm 5.97	163.80 \pm 4.97	162.22 \pm 3.84	173.92 \pm 1.28	p<0.05
	2	168.60 \pm 5.13	173.80 \pm 4.50	158.75 \pm 1.64	172.55 \pm 1.04	p<0.05
Fatty liver (%)	1	-	-	0.70 \pm 0.28	3.46 \pm 1.14	p<0.05
	2	-	-	2.71 \pm 1.74	2.88 \pm 2.77	p>0.05
Blood vessels*	1	-	-	24.00 (20.50-24.00)	32.00 (31.50-34.00)	p<0.05
	2	-	-	21.00 (17.50-28.50)	35.00 (31.00-38.50)	p>0.05

*express in median (IQR)

[#]p-value of average control vs. average treatment

Results on blood cholesterol level

For blood cholesterol measurement, the mean baseline data was found to be lower by 1.40 ± 3.48 mg/dL in treatment group of 1 month intervention compared to that of control group of 1 month intervention (163.80 ± 4.97 mg/dL vs. 165.20 ± 5.97 mg/dL, respectively). In contrast, the baseline cholesterol data was recorded to be less by 5.20 ± 3.05 mg/dL in the control group 2 than that of treatment group 2 (168.60 ± 5.13 mg/dL vs. 173.80 ± 4.50 mg/dL). Similar to the baseline data of blood glucose level, there were no significant differences among the baseline cholesterol data of control groups and treatment groups (independent t-test; p>0.05) (Table 2).

The cholesterol level decreased significantly by 2.98 ± 2.33 mg/dL when compared before and after intervention in control group 1 (165.20 ± 5.97 mg/dL to 162.22 ± 3.84 mg/dL; paired t-test; p<0.05). In contrast, the cholesterol level showed a significant increase of 10.12 ± 4.99 mg/dL after intervention in treatment group 1 (163.80 ± 4.97 mg/dL vs. 173.92 ± 1.28 mg/dL; paired t-test; p<0.05). In the control group 2, the mean cholesterol level decreased from 168.80 ± 5.13 mg/dL at baseline to 158.75 ± 1.64 mg/dL after experiment. A Wilcoxon Signed-Rank Test revealed that this difference was significant (p<0.05). The mean cholesterol level was also found to be decreased from 173.80 ± 4.50 mg/dL at baseline to 172.55 ± 1.04 mg/dL after 2-month intervention. According to Wilcoxon Signed-Rank Test, this decrease was found to be not statistically significant (p>0.05) (Table 2).

After intervention in the 1-month groups, blood cholesterol level was found to be higher in the treatment group by 11.70 ± 1.81 mg/dL compared to that of control group (173.92 ± 1.28 mg/dL vs. 162.22 ± 3.84 mg/dL). This difference was found

to be statistically significant (independent t-test; p<0.05). Likewise, mean cholesterol level was also found to be higher in treatment group compared to control group following 2-month intervention (172.55 ± 1.04 mg/dL vs. 158.75 ± 1.64 mg/dL). A Mann-Whitney Test found that this difference was significant (p<0.05) (Table 2).

Results on fatty liver percentage

In 1 month groups, the percentage of fatty liver was found to be higher in the treatment group by 2.76 ± 0.53 % compared to that of control group (3.46 ± 1.14 % vs. 0.70 ± 0.28 %; independent t-test; p<0.05). In contrast, the percentage of fatty liver was found to be slightly higher in treatment group 2 (2.88 ± 2.77 %) compared to that of control group 2 (2.71 ± 1.74 %). The difference was 0.16 ± 1.46 % and revealed to be not significant (independent t-test; p>0.05) (Table 2).

Results on thickening of blood vessels

After 1 month, the median of the thickening of blood vessel is 24.00 (IQR: 20.50-24.00) in the control group and 32.00 (IQR: 31.50-34.00) in the treatment group. A Mann-Whitney test revealed that the higher value found in the treatment group compared to that of control group was significant (p<0.05). Correspondingly, the median of the thickening of blood vessel was also higher in the treatment group compared to that of control group in the 2 months groups. Nonetheless, result from Mann-Whitney test shown that this difference was not significant (p>0.05) (Table 2).

Results on correlation between various variables

In general, fatty liver percentage had no association with blood glucose level. (Pearson's correlation; r= -0.07; N= 20; p>0.05). Based on data obtained from 1-month group, blood glucose level

and percentage of fatty liver are strongly related (Pearson's correlation; $r=0.841$; $N=10$; $p<0.05$). It means that the high level of blood glucose was followed by the high level of fatty liver and vice versa. However, there was weak negative association (Pearson's correlation; $r=-0.216$; $N=10$; $p>0.05$) between blood glucose level and percentage of fatty liver in 2-month intervention group.

Looking into all available data, the correlation between fat deposition in liver and cholesterol level in the blood was weak and positive (Spearman's correlation; $r_s=0.394$; $N=20$; $p>0.05$). Breaking down the data based on the intervention timeline, there was a strong association between blood cholesterol level and percentage of fatty liver according to results from 1-month intervention group (Pearson's correlation; $r=0.816$; $N=10$; $p<0.05$). This indicated that high blood cholesterol level tends to have high percentage of fatty liver and vice versa. Difference result was obtained from 2-month intervention group revealing that there was little or no association among blood cholesterol level and percentage of fatty liver though this result was shown to be of no significance (Spearman's correlation; $r_s=0.128$; $N=10$; $p>0.05$).

Judging from all 20 subjects, thickening of blood vessels had significant strong positive association with blood glucose (Spearman's correlation; $r_s=0.619$; $N=20$; $p<0.05$). Higher blood glucose tends to get more thickening of vessel wall. Blood glucose level was found to have a strong and positive relation to thickening of blood vessels in group 1. When the level of blood glucose rose, the amount of thickened blood vessels also rose (Spearman's correlation; $r_s=0.788$; $N=10$; $p<0.05$). On the other hand, group 2 results shown that there was weak positive association among these 2 variables. Nevertheless, this association was found to be not significant (Spearman's correlation; $r_s=0.375$; $N=10$; $p>0.05$).

Overall, thickening of blood vessel was strongly associated with blood cholesterol level. The trend observed was increased in blood cholesterol level had tendency to have more thickening of blood vessels (Spearman's correlation; $r_s=0.691$; $N=20$; $p<0.05$). In the 1-month group, there was a strong positive association among blood cholesterol level and thickening of blood vessels. This shown that high blood cholesterol level tended to have higher amount of thickened blood vessels (Spearman's correlation; $r_s=0.827$; $N=10$; $p<0.05$). In the 2-month group, the association between these

2 variables was found to be strong and positive (Spearman's correlation; $r_s=0.689$; $N=10$; $p<0.05$).

Discussion

Sprague Dawley rat was chosen as the subject because it was the only one available in the laboratory. This type of rat is the most widely used outbred rat in animal research as it has quiet and obedient disposition. Commonly used in toxicology, aging, teratology, oncology, nutrition and general studies, this rat also has excellent reproductive performance and maternal characteristics. The average age used in this experiment was 3 to 4 months as the rats start to get older and easier to feed.¹⁶

Global modernization has led to magnificent changes in eating pattern. People tend to eat more fast food and sweetened beverages as they are widely available, less time and money consuming while the tastes are still satisfactory. These fast foods and sweetened beverages usually contain high amount sugar and fat. Therefore, this experiment was done to reveal the effects of these excessive intakes to the body especially liver. Liver was chosen as it is the main organ for metabolism and storage. Therefore, calculation of fatty liver percentage and thickening of arterioles were also used as the outcomes.

The amounts of sugar and lard given in this study were 5 ml each in an alternative dosing which were subsequently given once every two days. 5 ml of sugar was equal to 10 mg. This amount was used based on the average weight of the rats that intervention was given with amount of 5% of the total body weight.^{14, 17} The sugar used in this research was table sugar which is the sugar commonly used in daily cooking. This type of sugar is made from sucrose and therefore the contents are 50% glucose and 50% fructose.¹⁷

From the result of the experiment, the blood glucose levels of all subjects before the commencement of the research were within normal limits as the highest value of blood glucose level at that point was 109 mg/dL. The measurement used in this experiment was random blood glucose and the normal range should be less than 200 mg/dL with blood glucose of 140-200 mg/dL indicates pre-diabetes. Through independent t-test, it can be concluded that the variation in blood glucose level at baseline in both control and treatment groups were not significant and therefore, changes occurred after that would have been less likely caused by the difference at baseline.

Proceeding to the intervention, the control groups had decreased level of blood glucose though these results were not significant. On the other hand, both of the treatment groups showed increasing blood glucose level with statistical significant. These suggest that the intervention given which in this case was alternate sugar and lard excess consumption have influence on blood glucose level. Further test strengthen these findings, statistically significant rise was also found when comparison between control and treatment groups were made. Odegaard et al¹⁸ also reported similar outcomes that consumption of fructose beverages causes higher blood sugar levels, visceral fat deposition and insulin resistance.

Blood cholesterol data before experiment was all in normal range with the highest reported value to be 180 mg/dL. The normal range of cholesterol was below 200 mg/dL. At baseline, the difference was not significant. After intervention, both control groups revealed significant decrease of blood cholesterol level. The treatment groups, on the other hand, had significant increased level of cholesterol in group 1 and non-significant decreased level of cholesterol in group 2. Comparisons were also made among control and treatment groups of the same timeline, results obtained were significant increase in all groups. From all the findings obtained, it can be concluded that blood cholesterol level increased with the intervention given. Nonetheless, the increase in cholesterol level was still classified as within normal limits. These outcomes was consistent with the study done by researchers from Emory University¹⁹ in 2010 that excessive sugars lead to increase LDL cholesterol that contribute to alteration in blood vessels increasing the risk of heart disease.

Fatty liver percentage was significantly increased in 1-month intervention groups but was not significantly increased in 2-month intervention groups. In a Danish study²⁰ where they compared the effects of sucrose-sweetened cola to the amount of visceral fat, they found that the sucrose-sweetened cola increase fats in liver and muscle more than double. They also mentioned that fructose-containing sugars lead to increase in liver fat. These findings of increase in liver at levels may trigger events that eventually lead to insulin resistance making the early steps towards diabetes and heart disease. Although the results found in this Sprague Dawley rat experiment were congruent with the outcomes from the Danish study, it could not be clearly distinguished as sugar in general was

used and not the specific type of sweeteners such as fructose or sucrose only. Study done by Neves et al²¹ also showed that high-fat diet induce fatty degeneration and hepatic steatosis. Both of these studies have congruent findings with this experiment that high sugar and fat diet induce hepatic steatosis.

In both timeline, the thickening of blood vessels were significant as obtained from Mann-Whitney test. These reveal that excessive consumption of sugar and lard influence the occurrence of fatty liver and abnormal thickening of blood vessels. In a study conducted by Fung et al²², sugar-sweetened beverages were reported to cause increase in inflammatory markers that affects plaque stability and thrombosis and eventually leading to atherosclerosis. However, their study used on female human as the subjects and obtained the results based on questionnaire evaluation. As a result, the result obtained from Fung et al²² study may not be applicable to this rats study due to remarkable difference between subjects and methods.

From all correlations test done, several general patterns can be drawn. Overall, blood glucose level showed little or no association with fat deposition in liver while blood cholesterol level was found to have weak and positive relationship with fatty liver percentage. Both blood glucose and cholesterol level have strong positive association with thickening of blood vessels. From these outcomes, conclusion could be made that high-fat diet, as measured using blood cholesterol level, had bigger influence to percentage of fatty liver and thus had higher chances of developing fatty liver disease. Whereas for arteriosclerosis, results found that both high-fat and high-sugar diet had similar influence.

Breaking down the results based on the research timeline, both high-fat and high-sugar diet had strong effects to fatty liver percentage and thickening of blood vessels in after 1 month of excessive intakes. With longer intakes of 2 months, only high-fat diet had been revealed to have impact on arteriosclerosis. Others variables showed either weak or no correlation to both fatty liver and arteriosclerosis. These were seen by the plateau trends observed when comparing 1-month and 2-month groups. Several reasons could explain this phenomenon including that over-time the body has adjusted to the changes and therefore higher dosage of sugar and fat are needed to exert the effects.

In this experiment, the sample size was smaller than the actual number indicated. This was due to

the death of subjects before the end of experiment and therefore was not included as part of the analysis. The small sample size may cause the inability to detect small changes. Other issues that could be regarded as confounding factors include daily food intake of the rats, stress factors and environmental factors. Daily food intake of the rats may also contain certain amount of sugar and fat. The exact percentage of these elements in the food intake was unknown, therefore the influence of this factor to the result could not be evaluated.

Conclusion

Increased intakes of sugar and fat have significant effects on the body including liver. Based on the results found in this experiment, fat deposition was found to increase by more than double in the intervention group after 1 month. Nevertheless, the result in 2 months intervention was mostly not significant. Further studies are still needed to assess other factors that may cause this incongruent result.

The amounts of thickened blood vessels were significant in both treatment groups compared to the controls. These findings congruent with studies done by other researchers that excessive sugar and fat consumptions could lead to changes in blood vessels, eventually may further progress to atherosclerosis.

Further studies are still needed to evaluate more on the exact effects of excess intakes of sugar and fat as several variables still presented with incongruent outcomes to the general patterns especially in the longer period of experiment. In addition, further studies should take notice regarding the sample size that was inadequate in this study.

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